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**Research Note**

# Screening Bovine Carcass Sponge Samples for *Escherichia coli* O157 Using a Short Enrichment Coupled with Immunomagnetic Separation and a Polymerase Chain Reaction–Based (BAX) Detection Step†

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## ABSTRACT

A bovine carcass sponge sample screening protocol for detecting *Escherichia coli* O157:H7 was composed of a short selective enrichment followed by an immunomagnetic separation (IMS) and target detection using the BAX *E. coli* O157 polymerase chain reaction assay. This screening protocol was compared to a culture-based method for detection of the organism in carcass sponge samples. Enriched samples were subjected to IMS; the bead suspension was divided and plated on selected media or stored at −20°C, then subjected to BAX analysis. The results showed a high degree of agreement between the plating method and the BAX system. Fifty-two of the 59 culture-positive samples were also positive using the BAX system (88.1% sensitivity). Of the 76 samples that appeared negative for the presence of *E. coli* O157:H7 by the culture method, 66 were determined as negative using the BAX system (86.8% specificity). Four of the 10 samples found negative by the initial culture method and positive by the BAX method were subsequently found to be culture positive upon reanalysis. Based on these data, the BAX system combined with a short, selective enrichment and IMS may be a rapid, reliable, and simple method to screen for *E. coli* O157:H7 in carcass sponge samples. Our data indicate that optimization and subsequent testing of this protocol for use as a carcass screening tool are warranted.



*Escherichia coli* O157:H7 has emerged as a foodborne pathogen of major public health concern in the United States *(1, 10, 11)* and may cause severe disease and death in humans *(2, 4)*. In a 1993 outbreak of hemolytic uremic syndrome, 0.9 to 4.3 CFU of *E. coli* O157:H7 per g of ground beef were detected in lots of the product *(2, 9)*. The U.S. Department of Agriculture Food Safety and Inspection Service enforces a regulation that declares that the presence of *E. coli* O157:H7 at or above 1 CFU/25 g of ground beef constitutes a dangerously adulterated product. As a result, several methods of detecting this pathogen have been developed and compared with strictly cultural techniques.

Johnson et al. *(9)* reported the BAX system of fast screening for *E. coli* O157:H7 in ground beef samples, and improvements to the methodology were recently introduced (advanced BAX system) to increase sensitivity *(8)*. The recommended method includes a 24-h enrichment step for food samples, followed by a proprietary polymerase chain reaction (PCR) amplification using prepackaged ingredients. This method has not been tested previously for detection of *E. coli* O157:H7 in carcass sponge samples.

No standard cultural method exists for recovery of *E. coli* O157:H7 from carcass sponge samples. Previously, researchers at the Roman L. Hruska U.S. Meat Animal Research Center used a newly developed culture method to determine the prevalence of *E. coli* O157:H7 or O157:nonmotile (*E. coli* O157:H7/NM) on carcasses *(5)*. That cultural detection method incorporated a 10-h selective enrichment, immunomagnetic separation (IMS) to recover and concentrate *E. coli* O157 cells and selective plating to discern suspected *E. coli* O157:H7/NM colonies. Colonies with the appropriate phenotype were screened with a commercially available diagnostic device. Selective plating required an overnight incubation period and skilled workers to identify colonies of the correct phenotype. The study reported here was designed to determine the efficacy of the advanced BAX system for rapidly screening the same short period-enriched and IMS-treated carcass samples for the presence of *E. coli* O157:H7, thereby eliminating the need for plating and colony identification during screening. Therefore, the recommended 24-h enrichment for the BAX system was, in effect, replaced with a shorter, selective enrichment and IMS concentration of the target organism.

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## MATERIALS AND METHODS

**Sample preparation.** Acquisition of the beef carcass samples has been described previously *(5)*. In brief, carcasses were sponge sampled across both inner and outer hocks and across the perineum immediately after complete hide removal and prior to evisceration. Samples collected in 25 ml buffered peptone water were enriched in 90 ml brilliant green bile broth 2% for 10 h at 37°C, followed by anti-O157 immunomagnetic bead enrichment and selective plating *(5)*. Colonies of the appropriate phenotype were screened with ImmunoCard Stat! (Meridian Diagnostics, Cincinnati, Ohio). Positive colonies were then streaked for isolation. Samples were considered to be positive only once isolated colonies were confirmed as *E. coli* O157:H7/NM by biochemical analyses, ELISA for the O157 and H7 antigens, and PCR to determine the presence of $rfb_{O157}$, *eae, stx1, stx2,* and *hlyA (5)*.

**Advanced BAX system.** The immunomagnetic bead suspensions were stored at −20°C and subsequently examined for *E. coli* O157:H7 by the advanced BAX system (Qualicon, Washington, Del.). Five microliters of the bead suspensions was mixed well in sterilized tubes with the supplied protease and lysis solutions. The samples were heated at 37°C for 20 min, then at 95°C for 10 min to inactivate the protease. The lysates were placed in a cooling block at 4°C for 5 min; then, 50 μl of the lysates was transferred into PCR tubes containing premixed reagents and subjected to amplification per the manufacturer's instructions. After thermal cycling, the reactants were gently mixed with 15 μl of supplied dye (Qualicon), and 15 μl of the mixtures was analyzed by agarose gel electrophoresis. Presence or absence of a band only of the specified size was recorded. This system includes positive and negative controls, which were run in conjunction with samples. The few samples providing indeterminate results, i.e., very weak bands, were reanalyzed. Since additional beads were no longer available, 5 μl of the BAX amplification reactions was subjected to an additional round of BAX amplification and analysis as described for enrichment samples. This procedure resulted in clear-cut identification of the presence or absence of a band.

**Additional culture steps.** Samples that were negative by the original culture method *(5)* and positive by the advanced BAX system were subjected to further analyses based on a variation of the method of Fukushima and Gomyoda *(6)*. One milliliter of the original enrichment, prior to IMS, had been stored with 12% glycerol at −70°C. These enrichments were gently thawed, and 0.8 ml was added to 9 ml trypticase soy broth (BBL, Cockeysville, Md.), then incubated at 42°C for 6 h. One milliliter of this culture was subjected to IMS and selective plating as before *(5)*, both with and without an acid shock step *(6)*.

Colonies with a phenotype consistent with *E. coli* O157:H7 were screened then tested with ImmunoCard Stat! (Meridian) for detecting the O157:H7 serotype.

## RESULTS AND DISCUSSION

A total of 135 carcass sponge sample selective enrichments were evaluated by the advanced BAX system. Table 1 shows a comparison of the advanced BAX system to the culture method. Fifty-nine of these samples were determined as *E. coli* O157:H7/NM positive (43.7%) by a culture method (Table 1) *(5)*. Isolates from each of these samples have been characterized in detail to confirm their identity as *E. coli* O157:H7/NM *(5)*. The sensitivity and specificity of the advanced BAX system compared to the culture method were 0.88 and 0.87, respectively, and the positive and negative predictive values were 0.84 and 0.90, respectively. These values are particularly notable in light of the high prevalence of *E. coli* O157:H7/NM isolation among the samples used in this study, i.e., the apparent sensitivity of the culture method. However, the Food Safety and Inspection Service has established guidelines of ≥98% sensitivity and ≤10% false-negative rate for ELISA-based screening tests used to check 20- to 24-h enrichments of ground beef and other samples *(3)*.

TABLE 1. *Detection of* E. coli *O157:H7 in carcass sponge samples by the advanced BAX system versus a previously described culture method (5)*

| | Culture method | | Advanced BAX total |
|---|---|---|---|
| | Positive | Negative | |
| Advanced BAX positive | 52 (38.5)[a] | 10 (7.4) | 62 (45.9) |
| Advanced BAX negative | 7 (5.2) | 66 (48.9) | 73 (54.1) |
| Culture method total | 59 (43.7) | 76 (56.3) | 135 (100) |

[a] Number of samples (percentage of total samples).

Ten out of 76 samples determined negative for *E. coli* O157:H7/NM by the culture method *(5)* were found positive by the advanced BAX system (Table 1). Enrichments stored frozen (−70°C) from these 10 samples were reanalyzed (see "Materials and Methods") in an attempt to isolate the organism. Four out of 10 of these samples were identified as positive samples after an additional enrichment and IMS procedure. Thus, the specificity and positive predictive value of the BAX system could be considered 0.92 and 0.90, respectively, congruent with Food Safety and Inspection Service guidelines for an ELISA-based screening assay *(3)*.

Fifty-two of the 59 culture-positive samples were positive by the advanced BAX system. One possible reason for false-negative samples and apparent lower sensitivity by the advanced BAX system is the dilution factor. Only 5 μl of the immunomagnetic bead suspension was effectively diluted and then analyzed in this system, whereas 50 μl of the bead suspension was plated in duplicate in the culture method *(5)*. Analysis of a larger bead sample by the BAX system might have improved the test sensitivity and decreased the rate of false negatives, but larger bead suspension volumes were not available. In addition, the advanced BAX system purportedly requires >6.0 log CFU/ml of *E. coli* O157:H7 in the enriched culture sample *(7)*, but the culture method theoretically could detect as little as 1 CFU/ml. While the population of *E. coli* O157:H7/NM in the enrichment might be expected to increase to sufficient levels for BAX detection under optimal conditions, nothing is known about the growth of the organism under enrichment conditions encountered by natural isolates in carcass sponge samples. It should be noted that the advanced BAX system does recommend a 24-h enrichment in the case of ground beef samples, whereas we included only a 10-h enrichment step. However, since 1 ml of enrichment was subjected to IMS and brought to a final volume of 100 μl, the 5-μl IMS aliquot could be considered similar to $1/20$ of 1 ml of enrichment, or 50 μl of enrichment. The BAX system rec-

ommendations for food products include a 24-h enrichment and use of 5 μl of the enrichment. While a longer enrichment time could allow for additional *E. coli* O157:H7 growth, IMS provided for a 10-fold increase in the effective volume of enrichment analyzed. Furthermore, a longer enrichment time would have substantially increased the analysis time, thereby eliminating some of the benefit of using the BAX system.

These data suggest that the previously described culture method, although apparently very sensitive, may have been unsuccessful at finding some positive samples and that the advanced BAX system can complement the culture method to identify these positive samples. Note that the culture method used to identify the positive samples is the only published method for analyzing carcass sponge samples. These data also suggest that the remaining six samples identified as negative by the culture method could have been falsely identified as positive by the advanced BAX system, or the organisms were missed by the culture methods. Recently published data detailing the performance of the BAX system suggest that false positives are highly unlikely *(7)*.

The advanced BAX system coupled with an abbreviated enrichment period and IMS took much less time compared to the previously described culture method for detecting *E. coli* O157:H7 in carcass sponge samples (approximately 24 versus 48 h). Johnson et al. *(9)* reported 96.5% correlation between the original BAX system and a culture method with 24-h enriched food samples, but under these circumstances, the time savings would be negated. In this study of carcass sponge samples, we used a 10-h enrichment period with IMS concentration of cells and obtained 88.1% agreement in the identification of positive samples between the advanced BAX system and the recently described culture method *(5)*. Given the time required and the technical difficulty of correctly identifying *E. coli* O157:H7 by the culture method, a simple, rapid, and reliable detection method is valuable for screening carcass sponge samples to detect *E. coli* O157:H7. The advanced BAX system used as a detection assay is user-friendly and demonstrates excellent specificity for preliminary screening of carcass sponge samples to detect *E. coli* O157:H7. These data warrant further optimization of the assay conditions, leading to an evaluation of this screening tool on a larger scale.

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